IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Girard, et al.
Appl. No.	:	10/539,527
Filed	:	July 10, 2006
For	:	**NF-HEV COMPOSITIONS AND METHODS OF USE**
Examiner	:	Shin, Dana H
Group Art Unit	:	1635

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF
DR. JEAN-PHILIPPE GIRARD UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. Jean-Philippe Girard, do hereby declare and state that:

1. I am a co-inventor of the subject matter described and claimed in U.S. Patent Application No. 10/539,527, filed on July 10, 2006 entitled, "NF-HEV COMPOSITIONS AND METHODS OF USE".

2. I am the Director of the Department of Cancer Biology and the Head of the Laboratory of Vascular Biology at the Institut de Pharmacologie et de Biologie Structurale, Toulouse, France. My curriculum vitae is attached as Exhibit A.

3. I have read and understand the specification of United States Patent Application No. 10/539,527. Additionally, I am familiar with the prosecution history of this patent application, including the subject matter of the currently pending claims.

4. I understand that the Examiner rejected the claims of the above-referenced patent application for allegedly failing to comply with the written description requirement and enablement requirement under 35 U.S.C. § 112, first paragraph.

5. I declare that recent studies have shown that NF-HEV exhibits structural homology with interleukins and induces the expression of numerous molecules involved in inflammation.

A. NF-HEV was renamed interleukin-33 (IL-33) in 2005 after Robert Kastelein and colleagues at DNAX/Schering Plough discovered that the C-terminal domain of NF-HEV (residues 112-270) exhibits structural homology with IL-1 family cytokines and can function as an IL-1-like ligand for the ST2 (IL-1R4) receptor (see, Schmitz, J. et al., *Immunity*, 2005, **23**:479-490).

B. There are approximately 20 recent papers that have confirmed the IL-1-like domain of NF-HEV/IL-33 (amino acids 112-270) activates the ST2 receptor, which is expressed on Th2 lymphocytes, mast cells, basophils, eosinophils, NK and NKT cells. Activation of the ST2 receptor on these various cell types with recombinant IL-33 (amino acids 112-270) has been shown to induce production of Th2-associated cytokines (IL-4, IL-5, IL-13) as well as pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IFNγ) and chemokines (CXCL8/IL8, CCL2/MCP1, CCL1).

6. I declare that, either directly or under my supervision, experiments were performed which demonstrate that the level or activity of NF-HEV polypeptide can be reduced by nucleic acid compounds, which in turn, reduce the level or activity of pro-inflammatory cytokines. The nucleic acid compound used in the experiments described herein was an siRNA molecule complementary to at least a portion of nucleic acid encoding NF-HEV. The following describes in detail methods of performing the experiments and the data obtained.

A. Methods for providing small interfering RNAs (siRNAs) against NF-HEV (IL-33) and measuring mRNA levels of NF-HEV (IL-33) and pro-inflammatory cytokines

Methods of reducing the activity of NF-HEV using small interfering RNAs (siRNAs) were performed as set forth in Example 20 of the instant application. The target gene was SEQ ID NO: 1, which contains the nucleic acid sequence encoding the elected NF-HEV polypeptide (SEQ ID NO: 4). In particular, the siRNAs tested in the present experiment were 21-basepair, double-stranded RNAs having complementarity to nucleobases 397-415 of SEQ ID NO: 1 (NF-HEV-siRNA#1); nucleobases 552-570 of SEQ ID NO:1 (NF-HEV-siRNA#2); nucleobases 1250-1268 of SEQ ID NO:1 (NF-HEV-siRNA#3); and nucleobases 1754-1772 of SEQ ID NO:1 (NF-HEV-siRNA#4). In addition, a negative control siRNA, which was not significantly complementary to SEQ ID NO: 1, was also tested. Each of the siRNAs was purchased from Dharmacon (Lafayette, CO).

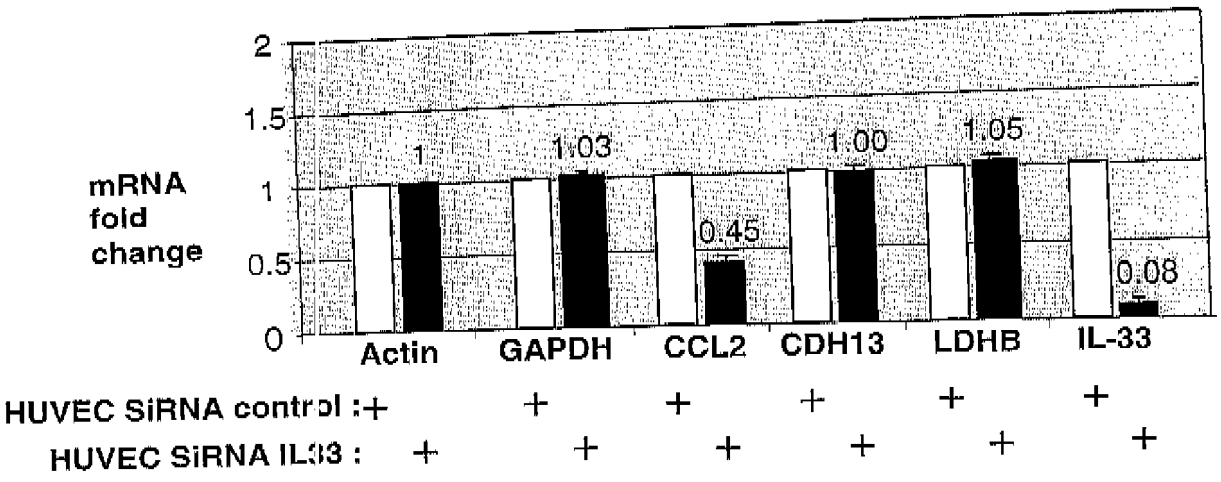
The ability of the NF-HEV siRNA to regulate the level of various mRNAs was tested in cultured human primary endothelial cells (HUVECs). Two successive transfections of HUVECs were performed at 24 hour-intervals by incubating the cells for 6 hours with the siRNA against NF-HEV (a mixture of the 4 NF-HEV-siRNAs) or the control siRNA at 50 nM final concentration in Oligofectamine and serum-free Opti-MEM-I (Invitrogen). RNA samples for quantitative PCR (qPCR) assays were taken 48 hours after transfection. Quantitative PCR was performed using an ABI7300 Prism SDS Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA) with a SYBR Green PCR Master Mix kit (Applied Biosystems) and a standard temperature

protocol according to the manufacturer's instructions. *Actin* was used as a control gene for normalization. QuantiTect Primer Assays (Qiagen) were used for analysis of indicated genes with their specific primers (control gene: *GAPDH, cadherin H/CDH13 and Lactate deshydrogenase/LDHB*). The mean and standard error for three separate data sets are shown in Figure 1. The results are representative of two independent experiments.

B) Results showing that siRNA targeting NF-HEV (IL-33) nucleic acid specifically and effectively reduces the level of mRNA of NF-HEV (IL-33), which in turn, reduces the activity or level of pro-inflammatory cytokines

Figure 1 shows the mRNA level of multiple genes in the absence or presence of the NF-HEV siRNAs. The white bar represents the mRNA level of each indicated gene with the control siRNA treatment, whereas the black bar represents the relative mRNA level of the genes with the NF-HEV siRNA treatment. The value of each black bar indicates percent knock-down of mRNA expression as compared to the control siRNA treatment. The mRNA level of control genes, such as *Actin, GAPDH, CDH13* and *LDHB*, is substantially unchanged by transfection of the NF-HEV siRNAs. However, mRNA of NF-HEV (IL-33) was significantly reduced, by more than 90%, in the presence of its specific siRNAs. As the amount of protein produced depends on the level of its nucleic acid, this 90% or more reduction of NF-HEV mRNA by the siRNA treatment substantially decreases the amount of NF-HEV protein that can be made. This result indicates that nucleic acid compounds, such as siRNA, targeting at least a portion of NF-HEV are effective inhibitors of NF-HEV expression. Of further significance is the fact that siRNA to NF-HEV also knocked-down the production of the inflammatory chemokine, CCL2/MCP-1, by over half. This shows that reductions in NF-HEV levels or activity also result in a reduction in the level or activity of pro-inflammatory chemokines.

FIGURE 1



7. I declare that the above-described experiment demonstrates that inhibition of NF-HEV can markedly reduce the level or activity of pro-inflammatory chemokines. As discussed above, the mRNA level of a pro-inflammatory chemokine, CCL2/MCP-1, was knocked-down by over 50% in response to NF-HEV siRNAs. In further support of the result that CCL2 transcription is affected by NF-HEV levels or activities, the instant application shows that CCL2/MCP-1 was identified as a target gene of NF-HEV using microarray assays (see Example 18). Moreover, we have shown that the expression of NF-HEV in cultured cells induced the expression of numerous pro-inflammatory chemokines, including CCL2/MCP-1 (see Example 19 of the instant specification).

8. In accordance with the foregoing experimental data, NF-HEV is predicted to be a transcription factor regulating the process of mRNA synthesis (see Figure 1, paragraph [0170] and Example 9 of the instant specification). As such, the transcription of pro-inflammatory cytokines can be reduced by reducing the level or activity of NF-HEV, which in turn, leads to a reduction in inflammatory symptoms.

9. I further declare that all statements made herein of knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/27/08
Date

Jean-Philippe Girard, Ph.D.

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